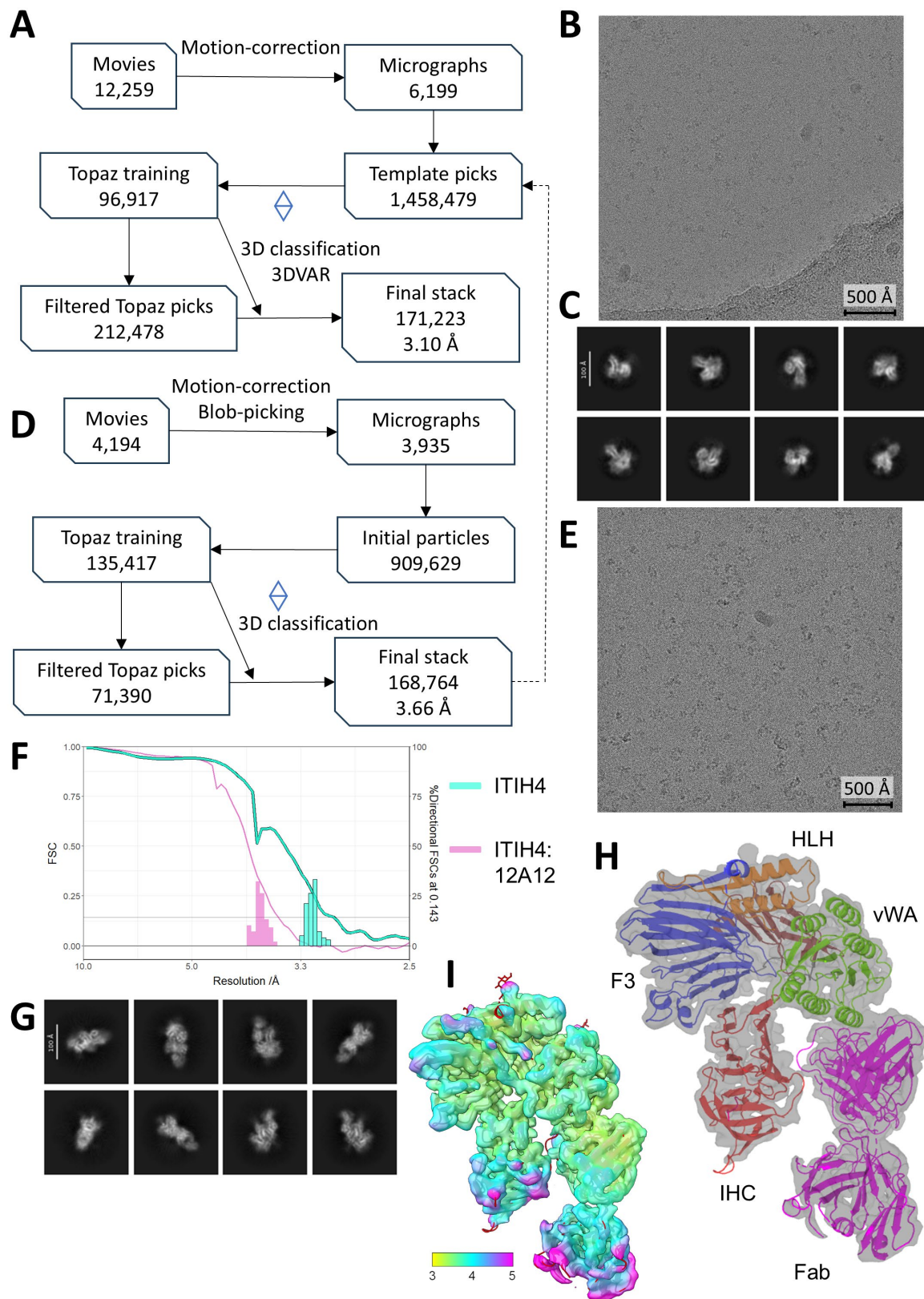


Supplementary information

Supplementary Figures

Supplementary Figure 1. Cryo-EM analysis of native ITIH4 and ITIH4:Fab. **A)** Flow-chart of single particle analysis (SPA) processing steps of ITIH4 showing particle number in the stack at each step. The blue rhombus means that the particles were filtered by 2D classification, ab-initio reconstruction and heterogeneous refinement. The templates for template picking were derived from the ITIH4:Fab final reconstruction. **B)** Representative motion-corrected micrograph of ITIH4 sample at a defocus of 1.5 μm . **C)** Representative 2D classes of ITIH4. **D)** Flow-chart as in A but for the ITIH4:Fab dataset. **E)** Representative motion-corrected micrograph of ITIH4:Fab sample at a defocus of 1.5 μm . **F)** Gold-standard (GS) Fourier shell correlation (FSC) curves for ITIH4 and ITIH4:Fab with directional FSC resolutions at 0.143 shown as histograms. **G)** Representative 2D classes of ITIH4:Fab. **H)** Model of ITIH4:Fab in the cryo-EM map shown as a transparent surface. **I)** The cryo-EM map of ITIH4:Fab coloured according to local resolution and overlaid with the model shown as a red cartoon.

Mechanism of ITIH4 activation and oligomerization

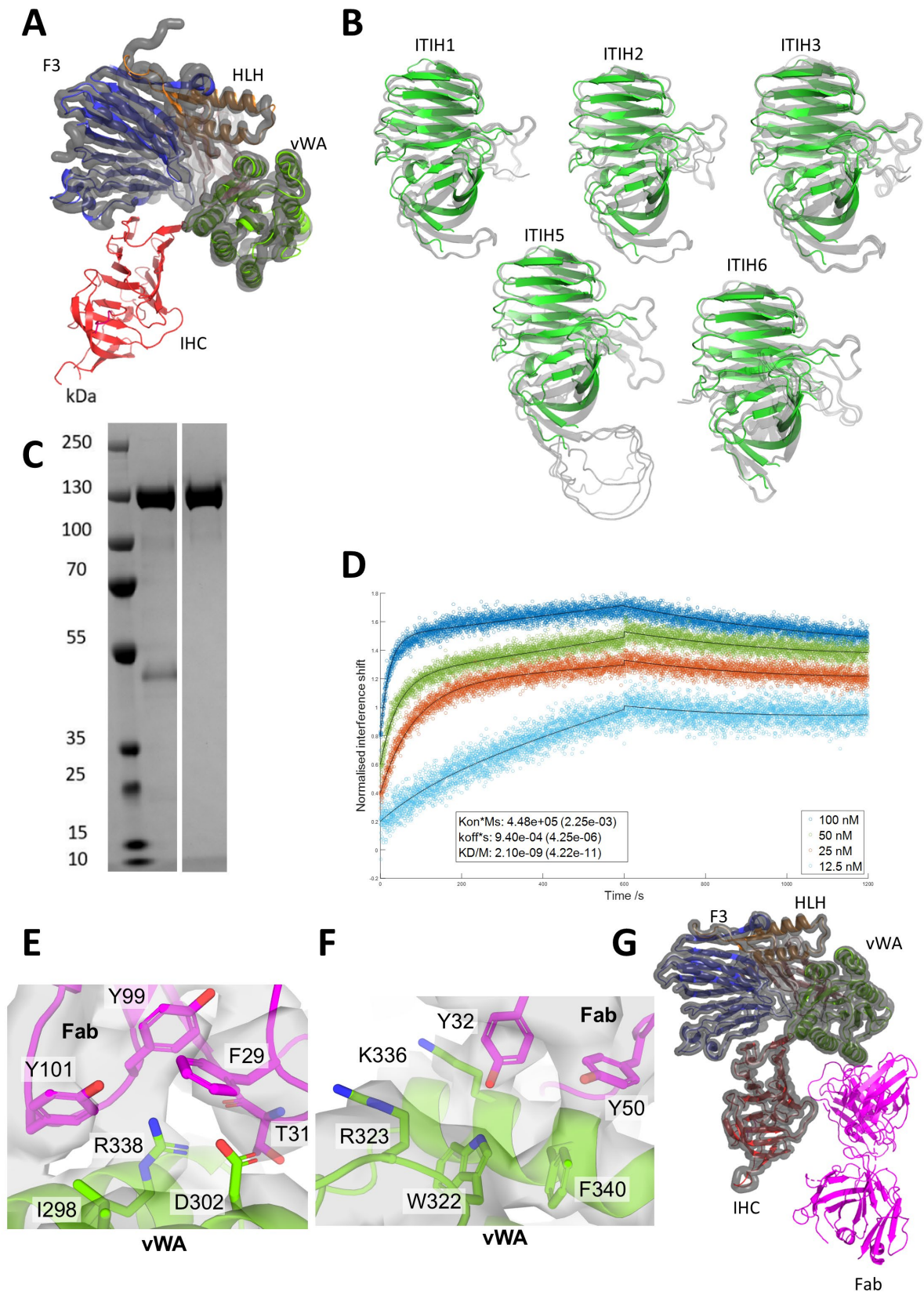


[illegible]

Supplementary Figure 3. Comparison of ITIH4 with other ITIHs and of the 12A12 Fab.

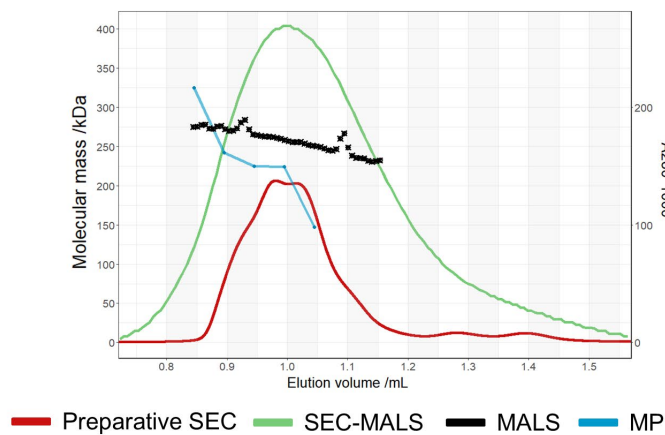
A) Cartoon representation of ITIH4 coloured as in Figure 1D and overlaid with the structure of ITIH1 (PDB entry 6FPY) shown as a thick, transparent cartoon in grey. While the models overall superpose very well, details around the F3 lid and TR differ, see also Figure 6C. **B)** Alphafold predictions (5 models) of the IHC of the other ITIHs shown as transparent, grey cartoons with the ITIH4 IHC shown in green superimposed. **C)** SDS-PAGE analysis of the ITIH4:Fab sample (left) and the ITIH4 sample (right) used for cryo-EM analysis. No clear degradation of ITIH4 is seen, and only the 50 kDa Fab band is seen apart from the 120 kDa ITIH4 band (glycans lead to the full-length 120 kDa ITIH4 running as 130 kDa marker on this gel). Since each cryo-EM data collection was only performed one time, the SDS-PAGE analysis of the samples used was performed once. **D)** BLI curves of 12A12 Fab binding to ITIH4, showing $K_D \sim 2$ nM. Performed once. **E-F)** The binding interface between the heavy chain (**E**) and the light chain (**F**) of 12A12 Fab in magenta cartoon and ITIH4 vWA in green and the cryo-EM map as transparent isosurface. **G)** Superposition of ITIH4 (grey, thick cartoon) and ITIH4:Fab (coloured as in Figure 1D), showing that the binding of the 12A12 Fab does not alter the conformation of ITIH4.

Mechanism of ITIH4 activation and oligomerization

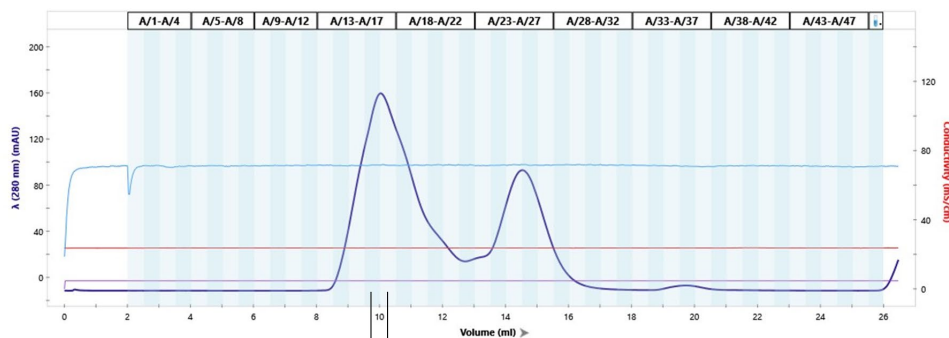


Supplementary Figure 4. MASP-1cf:ITIH4N complexes are very stable over time. **A)** The chromatogram from figure 2B (red) compared with a chromatogram obtained by SEC analysis of a similarly prepared pool from a larger amount of starting material applied to the same column but connected to a SEC-MALS detector on a distinct chromatography instrument (green). The peak broadening is caused by a longer flow-path in the SEC-MALS system. The average molecular mass estimate from MALS is shown as a black trace while the weighted average from peak picking in the mass photometry histograms for each fraction is shown as blue, connected dots. **B)** SEC chromatogram of ITIH4 incubated with MASP-1cf in a 5:2 ratio for 15 min at 37 °C. **C)** Fraction 17 from panel A applied to the same SEC column after 1 hour at 4 °C. **D)** Fraction 16 from panel A applied to the same SEC column after 12 days at 4 °C. An estimated 5% of protein has dissociated to smaller components. Experiments were performed once.

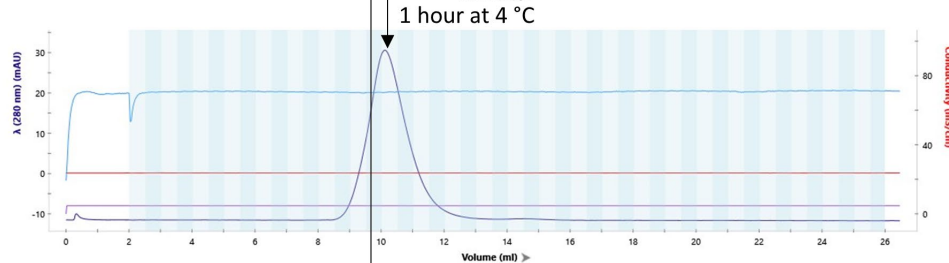
A



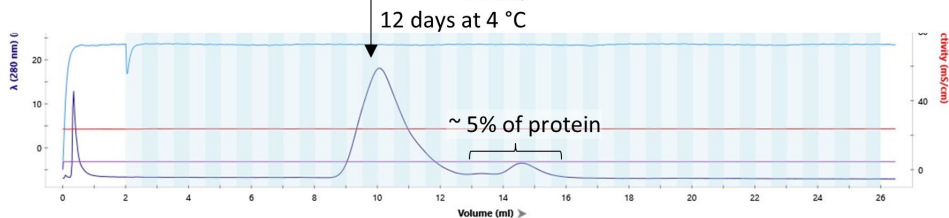
B



C

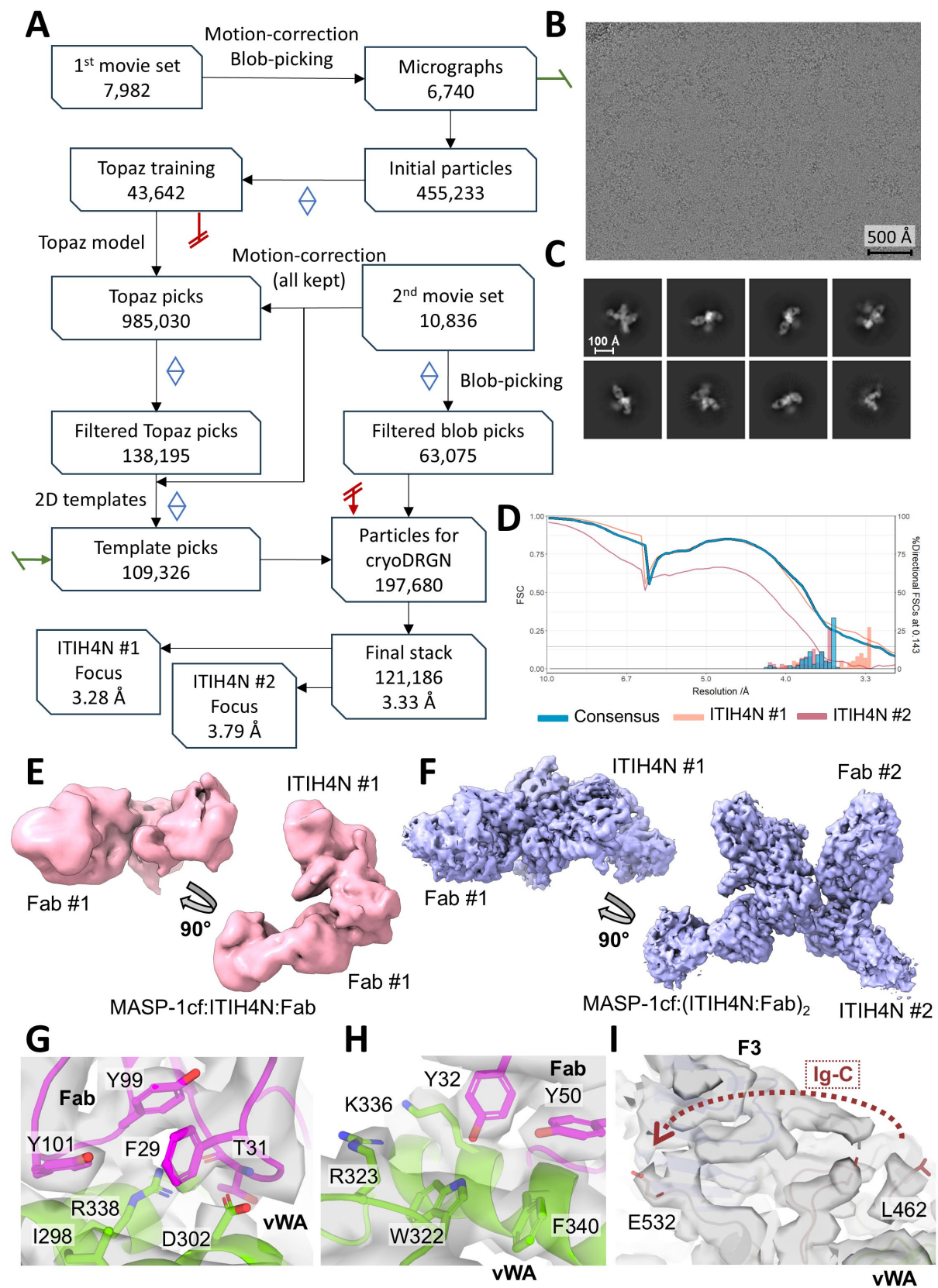


D

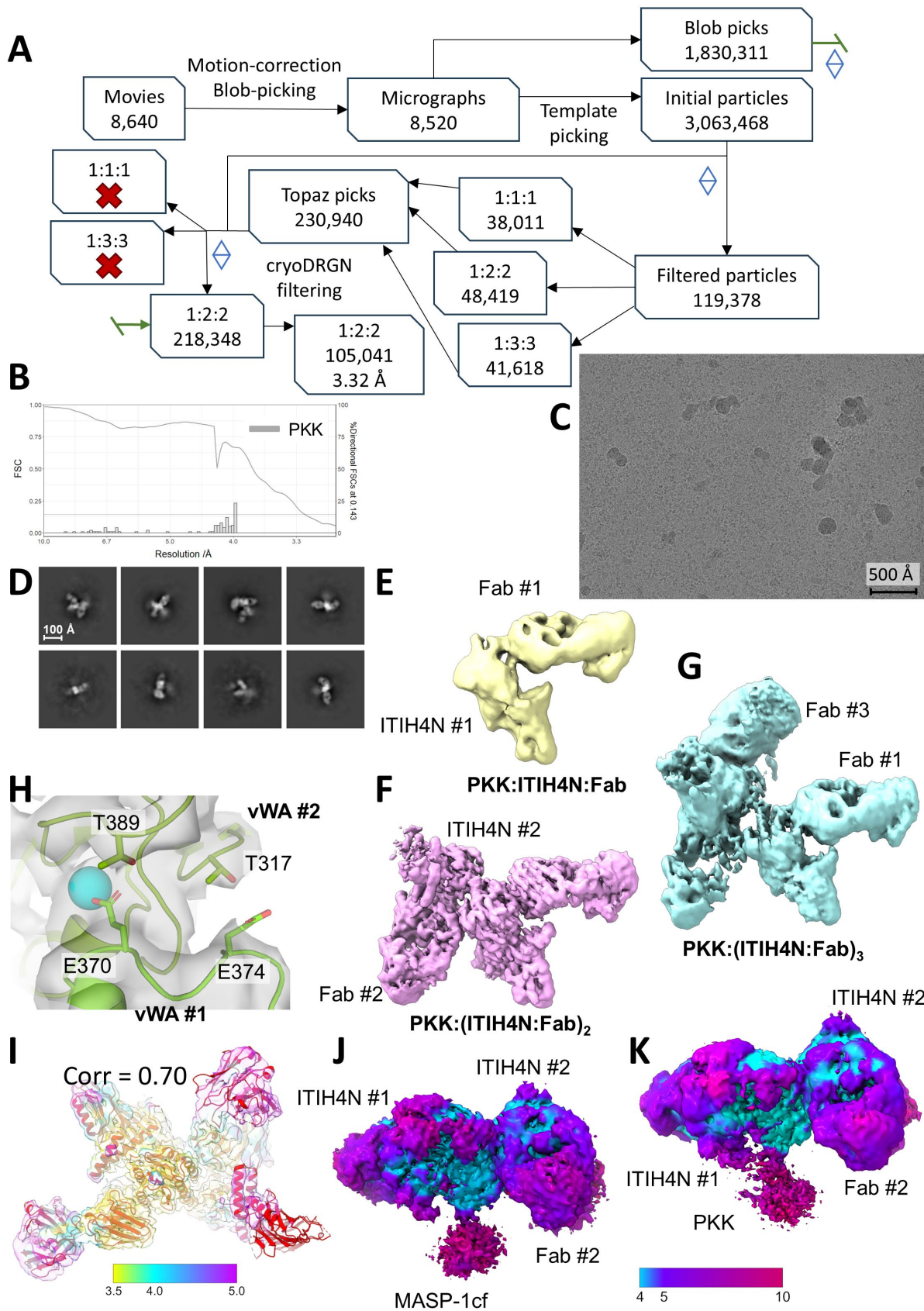


Supplementary Figure 5. Cryo-EM analysis of MASP-1cf:ITIH4N:Fab. **A)** Flow-chart of SPA processing steps of ITIH4N:MASP-1cf:12A21 showing particle number in the stack at each step. The blue rhombus means that the particles were filtered by 2D classification, ab-initio reconstruction and heterogeneous refinement. The interrupted lines in green and red continue at the arrow with the same colour. **B)** Representative micrograph of the MASP-1cf:ITIH4N:Fab sample at a defocus of 1.5 μm . **C)** Representative 2D classes. **D)** GS-FSC curve for MASP-1cf:(ITIH4N:Fab)₂ with directional FSCs at 0.143 shown as histograms. **E-F)** 3D classes obtained during processing, showing the 1:1:1 MASP-1cf:ITIH4N:Fab reconstruction that did not reach high resolution (E) and the MASP-1cf:(ITIH4N:Fab)₂ that reached a consensus resolution of 3.33 Å (F). **G-H)** The binding interface between the heavy chain (**G**) and the light chain (**H**) of 12A12 Fab in magenta cartoon and ITIH4N vWA in green and the cryo-EM map as transparent surface. **I)** The cryo-EM map of MASP-1cf:(ITIH4N:Fab)₂ is shown as a transparent surface with the model superposed and coloured as in Figure 1D. The lack of signal for Ig-C between L462 and E532 is marked with a dashed line.

Mechanism of ITIH4 activation and oligomerization

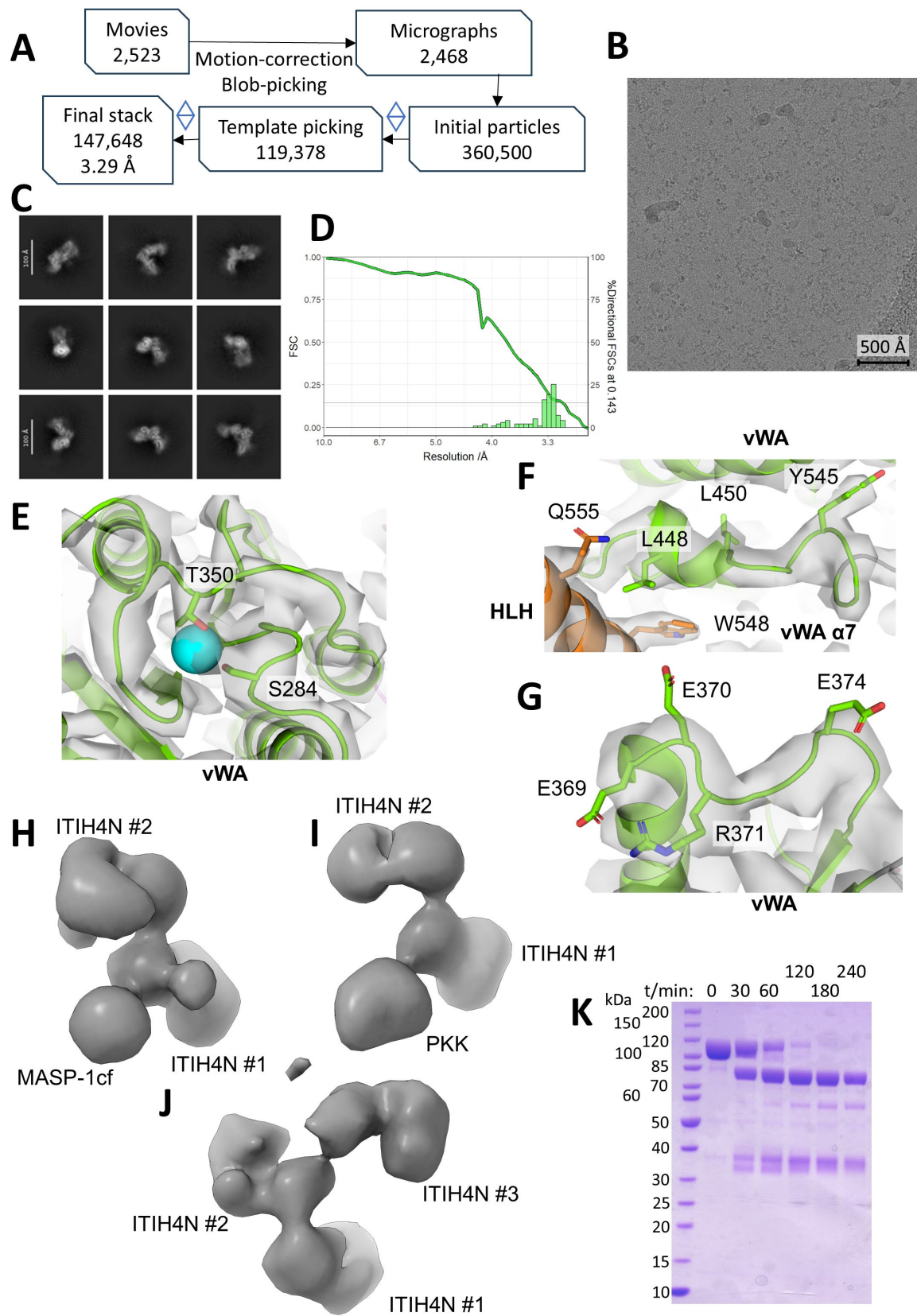


Supplementary Figure 6. Cryo-EM analysis of PKK-1cf:ITIH4N:Fab. **A)** Flow-chart of SPA processing steps of PKK:ITIH4N:Fab showing particle number in the stack at each step. The blue rhombus means that the particles were filtered by 2D classification, ab-initio reconstruction and heterogeneous refinement. The interrupted line in green continues at the arrow with the same colour. The red crosses at the final stages for 1:1:1 and 1:3:3 PKK:(ITIH4N:Fab) signify that no interpretable 3D reconstruction could be made due to insufficient orientational sampling. **B)** GS-FSC curve for PKK:(ITIH4N:Fab)₂ with directional FSCs at 0.143 shown as histograms. **C)** Representative motion-corrected micrograph of the ITIH4N:PKK:Fab sample at 1.5 μm defocus. **D)** Representative 2D classes. **E-G)** 3D classes obtained during processing, showing the 1:1:1 PKK:ITIH4N:Fab complex (E), PKK:(ITIH4N:Fab)₂ (F) and PKK:(ITIH4N:Fab)₃ (G). **H)** The model of MASP-1cf:(ITIH4N:Fab)₂ showing the interaction between E370 in ITIH4N#1 with the MIDAS of ITIH4N#2 overlaid with the cryo-EM map from PKK:(ITIH4N:Fab)₂. **I)** The model of MASP-1cf:(ITIH4N:Fab)₂ fitted into the PKK:(ITIH4N:Fab)₂ map, showing a correlation coefficient of 0.70 at a resolution of 3.3 Å, while the MASP-1cf:(ITIH4N:Fab)₂ correlates with its own map with a coefficient of 0.73 at the same resolution. The map is coloured according to local resolution. **J-K)** Cryo-EM map of MASP-1cf:(ITIH4N:Fab)₂ (J) and PKK:(ITIH4N:Fab)₂ (K) at low threshold and coloured according to local resolution. At this threshold, noisy electrostatic potential (ESP) is visible for MASP-1cf and PKK.

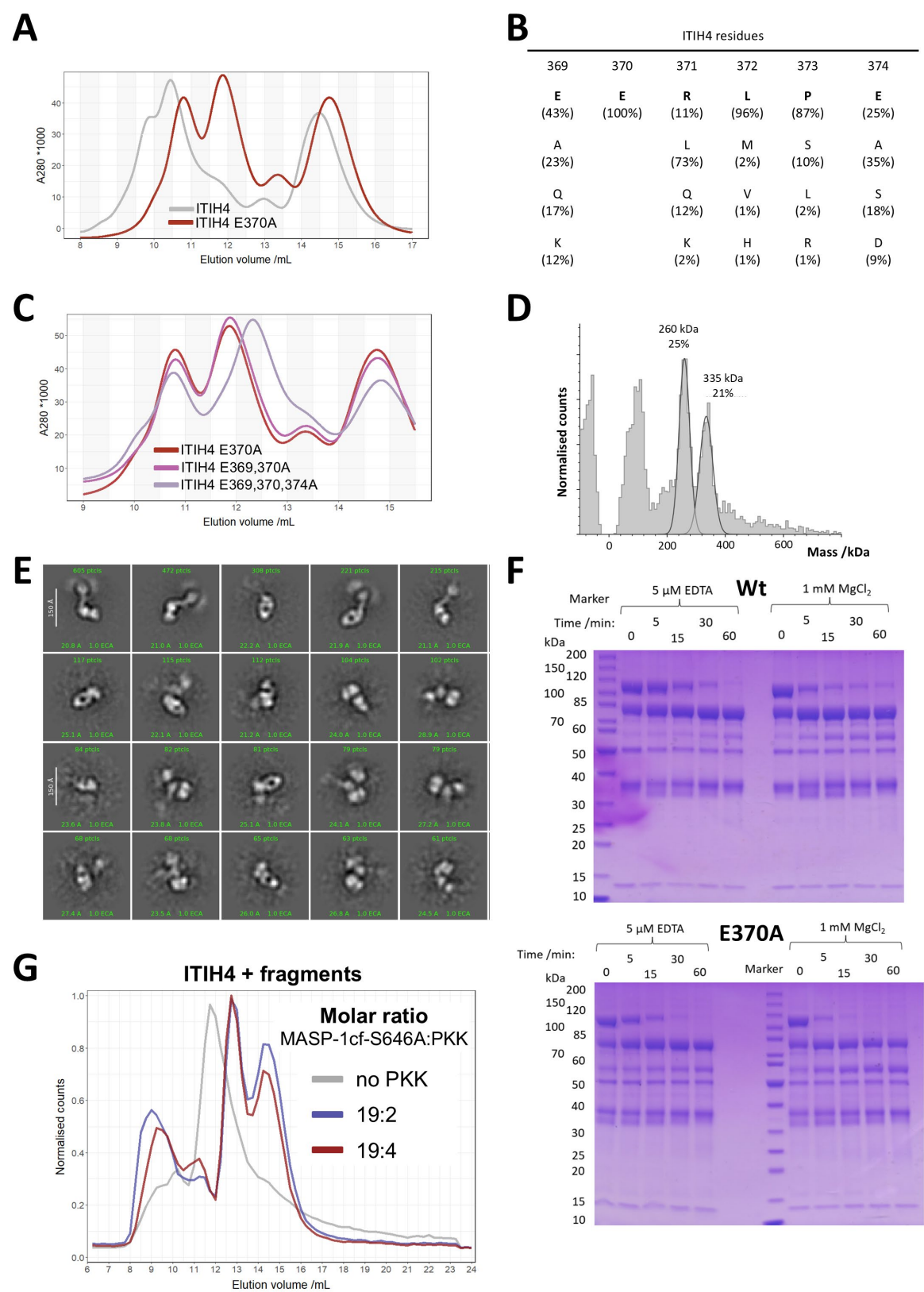


Supplementary Figure 7. Cryo-EM analysis of ITIH4N:Fab. **A)** Flow-chart of the SPA processing steps of the ITIH4N:Fab sample. The blue rhombus means that the particles were filtered by 2D classification, ab-initio reconstruction and heterogeneous refinement. **B)** Representative motion-corrected micrograph of the ITIH4N:Fab sample at 1.5 μm defocus. **C)** Representative 2D classes of ITIH4N:Fab. **D)** GS-FSC curve for ITIH4N:Fab with directional FSCs at 0.143 shown as histograms. **E)** Model of ITIH4N:Fab showing the Mg^{2+} in the MIDAS site overlaid with the cryo-EM map as a transparent surface. **F-G)** Model of ITIH4N:Fab overlaid with the cryo-EM map, showing the distortion of vWA $\alpha 7$ (F) and the conformational change of the loop containing E370 (G) as in MASP-1cf:(ITIH4N:Fab)₂. **H-J)** Envelopes from nsEM showing that subsequent ITIH4N fragments in a complex attach in a helical manner to the previous ITIH4N for both MASP-1cf:(ITIH4N)₂ (H), PKK:(ITIH4N)₂ (I) and PKK:(ITIH4N)₃ (J). **K)** Cleavage assay of ITIH4 with MASP-1cf in the same buffer and ratio as used for the circular dichroism measurements. The reaction is complete after 240 minutes. This experiment was performed once.

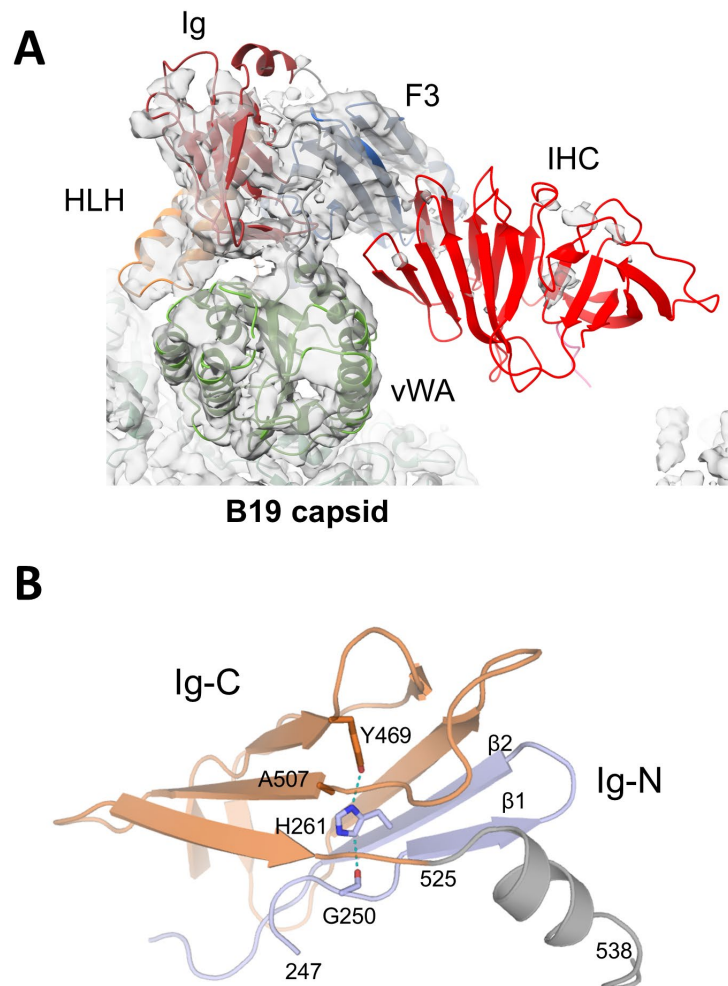
Mechanism of ITIH4 activation and oligomerization



Supplementary Figure 8. Mutation of conserved E370 abolishes homo-oligomerisation. **A)** SEC chromatogram of sample of MASP-1cf incubated with either ITIH4 (grey) or ITIH4^{E370A} (red) in a 3:1 ITIH4:MASP-1cf ratio for 15 min at 37 °C. While large complexes are strongly reduced for ITIH4^{E370A}, complexes larger than 1:1 are still formed. **B)** Sequence conservation among 186 unique, mammal sequences for ITIH4. The top row in bold shows the human sequence, while the rows below show the three most common residues apart from the human. Only E370 is fully conserved. **C)** Comparison as in panel A but for ITIH4^{E370A} (red), ITIH4^{E369,370A} (purple) and ITIH4^{E369,370,374A} (violet). **D)** Mass photometry histogram showing the 5F5:MASP-1cf:(ITIH4^{E370A}N)₂ (~260 kDa) and 5F5:MASP-1cf:(ITIH4^{E370A}N)₃ (~335 kDa) complexes present in the sample analysed by nsEM. **E)** Subset of 2D classes for 5F5:MASP-1cf:ITIH4^{E370A}N from nsEM SPA. The classes demonstrate that the particles are structurally very heterogeneous. **F)** Cleavage assay of ITIH4 (top) or ITIH4^{E370A} (bottom) with MASP-1cf in a 3:1 molar ratio at 37 °C in the presence of 5 µM EDTA (left) or 1 mM MgCl₂ (right). A similar result was observed in three replicates for the wild-type, while the experiment was performed once for ITIH4^{E370A}. **G)** The amount of ITIH4 or its cleavage fragments in fractions from SEC after incubation with a molar ratio of 11:19:0, 11:19:2 or 11:19:4 of ITIH4 to MASP-1cf-S646A to active PKK. Overall, shifts from full-length ITIH4 to larger complexes and smaller fragments are seen as in Figure 2A.



Supplementary Figure 9. ITIH4 binds parvovirus B19 in intermediate state. **A)** Overlay of ITIH4 model in cartoon representation coloured as in Figure 1D onto the map of ITIH4 bound to parvovirus B19 (EMD-45184). While ITIH4 is clearly in the native state based on the configuration of vWA, HLH and F3, no map is visible for the IHC domain. **B)** Cartoon representation of ITIH4 Ig domain with Ig-N in blue and Ig-C in orange. H261 is shown in sticks along with the neighbouring G250, Y469 and A507 residues.



Supplementary Tables

Data collection and processing	ITIH4	ITIH4:Fab	ITIH4N:Fab
Microscope	Titan Krios G4i	Glacios	
Camera	Falcon 4i	Falcon 4i	
Voltage (kV)	300	200	
Calibrated pixel size (Å)	0.748	0.714	
Defocus range (μm)	0.5-2.0	0.5-2.5	0.5-2.0
Exposure on sample (e ⁻ /Å ²)	70.0		
Dose fractions	EER		
Exposure time (s)	7.46	5.16	
Collected movies	12,259	4,194	2,523
Micrographs used for picking	6,199	3,935	2,468
Particle picks	1,458,479	909,629	360,500
Particles in final stack	171,223	168,764	147,648
Global resolution at FSC = 0.143 (Å)	3.02	3.66	3.29
Refinement			
Initial models	AlphaFold	AlphaFold + MASP-1cf:(ITIH4N:Fab) ₂	MASP-1cf:(ITIH4N:Fab) ₂
Model resolution masked/unmasked (Å)	3.3/3.4	4.0/4.1	3.6/3.8
FSC threshold	0.5	0.5	0.5
Model composition			
Atoms	6213	9386	7056
Protein residues	733	1188	898
Ligands	8	8	5
B factors (Å ² , min/max/mean)			
Protein	67.8/255.7/123.4	100.8/419.1/200.8	61.7/228.8/122.0
Ligand	90.1/179.5/135.2	77.7/279.5/203.8	120.2/195.6/166.2
R.m.s. deviations			
Bond lengths (Å)	0.005	0.005	0.005
Bond angles (°)	1.178	1.156	1.226
Validation			
MolProbity score	1.20	1.0	0.98
Clashscore	2.18	0.65	0.93
Poor rotamers (%)	0.59	0.19	1.02
CaBLAM outliers (%)	3.42	2.76	1.39
Ramachandran plot			
Favored (%)	96.74	95.83	96.82
Allowed (%)	3.26	4.17	3.18
Disallowed (%)	0.0	0.0	0.0
Model vs. Data			
CC (mask)	0.83	0.85	0.82
CC (box)	0.81	0.90	0.84
CC (peaks))	0.74	0.79	0.76
CC (volume)	0.83	0.85	0.82

Supplementary Table 1. Cryo-EM data collection, map and model refinement and validation statistics for the ITIH4, ITIH4:Fab and ITIH4N:Fab. Generated with PHENIX.

Mechanism of ITIH4 activation and oligomerization

Data collection and processing	MASP-1cf:(ITIH4N:Fab) ₂			PKK:(ITIH4N:Fab) ₂
Microscope	Titan Krios G3i			
Camera	Gatan K3			
Voltage (kV)	300			
Calibrated pixel size (Å)	0.647			
Defocus range (μm)	0.5-2.3			0.8-2.2
Exposure on sample (e ⁻ /Å ²)	59.6			
Dose fractions	53			
Exposure time (s)	1.4			
Collected movies	7,982 + 10,836			8,640
Micrographs used for picking	6,740 + 10,836			8,520
Particle picks	455,233 + 1,048,105			3,063,468
Particles in final stack	121,186			105,041
Map focus	Consensus	ITIH4N#1	ITIH4N#2	
Global resolution at FSC = 0.143 (Å)	3.33	3.28	3.79	3.32
Refinement				
Initial models	AlphaFold + PDB: 6WEQ			-
Model resolution masked/unmasked (Å)	3.9/4.0			
FSC threshold	0.5			
Model composition				
Atoms	14312			
Protein residues	1819			
Ligands	12			
B factors (Å ² , min/max/mean)				
Protein	48.9/336.9/155.8			
Ligand	104.8/214.2/174.1			
R.m.s. deviations				
Bond lengths (Å)	0.005			
Bond angles (°)	1.130			
Validation				
MolProbity score	1.29			
Clashscore	1.69			
Poor rotamers (%)	1.14			
CaBLAM outliers (%)	2.33			
Ramachandran plot				
Favored (%)	95.36			
Allowed (%)	4.64			
Disallowed (%)	0.0			
Model vs. Data				
CC (mask)	0.78			
CC (box)	0.84			
CC (peaks))	0.71			
CC (volume)	0.77			

Supplementary Table 2. Cryo-EM data collection, map and model refinement and validation statistics for MASP-1cf:(ITIH4N:Fab)₂ and PKK:(ITIH4N:Fab)₂. Since the model is not deposited for the PKK complex, model validation statistics are not given. Generated with PHENIX.

Mechanism of ITIH4 activation and oligomerization

Domain 1	Domain 2	ITIH4	ITIH4N
F3	Ig	1043	770
F3	vWA	512	0
F3	HLH	1896	1954
F3	TR	2412	2073
F3	IHC	1248	0
Ig	vWA	1217	1362
Ig	HLH	1773	467
Ig	TR	0	0
Ig	IHC	114	0
vWA	HLH	411	836
vWA	TR	160	0
vWA	IHC	518	0
HLH	TR	802	866
HLH	IHC	0	0
TR	IHC	0	0

Supplementary Table 3. Buried surface areas ($\text{\AA}^2$) at domain interfaces in native ITIH4 and the activated ITIH4N.